CERTIFICATE

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Bifidobacterium longum as a delivery system for cancer gene therapy: Selective localization and growth in hypoxic tumors

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A fundamental obstacle in gene therapy for cancer is the specific delivery of an anticancer gene product to a solid tumor, and yet no systemic delivery system that specifically targets solid tumors currently exists. A strain of domestic bacteria, *Bifidobacterium longum*, which is nonpathogenic and anaerobic, selectively localized and proliferated in several types of mouse solid tumors after systemic application. In this report, we further describe a novel approach to cancer gene therapy in which genetically engineered *Bifidobacterium* is used as a tumor-specific vector. Similarly to wild-type *B. longum*, genetically engineered *B. longum* could be detected in tumor tissue only and was not found in a large survey of normal mouse tissues after intravenous injection. This finding strongly suggests that obligate anaerobic bacteria such as *Bifidobacterium* can be used as highly specific gene delivery vectors for cancer gene therapy. Cancer Gene Therapy (2000) 7, 269–274

Key words: Bifidobacterium longum; *anaerobic bacteria; vector; cancer gene therapy; tumor targeting; hypoxia.*

Hypoxic regions are characteristic of solid tumors in rodents[1] and occur with high frequency in many types of human tumors.[2] Tissue oxygen electrode measurements taken in cancer patients show a median range of oxygen partial pressure of 10–30 mmHg in tumors, with a significant proportion of readings below 2.5 mmHg, whereas those in normal tissues range from 24 to 66 mmHg.[3] Gene therapy in solid tumors that targets gene expression to hypoxic tumor cells is currently being investigated.[4]

It is known that certain species of anaerobic bacteria, including the genera *Clostridium* and *Bifidobacterium*, can selectively germinate and grow in the hypoxic regions of solid tumors after intravenous (i.v.) injection.[5,6] The genera *Bifidobacterium* and *Lactobacillus* are Gram-positive anaerobes and are domestic, nonpathogenic bacteria found in the lower small intestine and large intestine of humans and other animals.[7–9] These intestinal organisms have been believed to have health-promoting properties for their host, including an increase of the immune response,[10] inhibition of carcinogenesis,[11] and protection of the host against viral infection.[12] However, despite the increasing attention to these bacteria in the fields of food science, medicine, and industry, little is known about their genetic properties, mainly due to the lack of efficient and reproducible systems for genetic transfer and adequate selectable markers, especially with regard to the genus *Bifidobacterium*. Recently, an *Escherichia coli-B. longum* shuttle vector has been constructed.[13]

We propose an innovative approach to cancer gene therapy in which genetically engineered anaerobic bacteria of the genus *Bifidobacterium* are used to achieve tumor-specific gene delivery.

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MATERIALS AND METHODS

Animals

Male C57BL/6 mice (Japan SLC, Hamamatsu, Japan) of 6 to 8 weeks of age were used in this study. Mice were fed a standard rodent diet (Oriental Yeast Company, Tokyo, Japan) in the Shinshu University animal center.

Tumors

B16-F10 melanoma cells and Lewis lung cancer cells were maintained as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO_2. A total of 5×10^5 tumor cells were inoculated into the right thigh muscle of these mice. The solid tumors obtained 2 weeks after inoculation were then used for study.